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Strategies for Eliciting HIV-1 Inhibitory Antibodies

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Abstract

Purpose of review—Major roadblocks persist in the development of vaccines that elicit potent neutralizing antibodies targeting diverse HIV-1 strains, similar to known broadly neutralizing HIV-1 human monoclonal antibodies. Alternatively, other types of anti-HIV-1 envelope antibodies that may not neutralize HIV-1 in traditional neutralization assays but have other anti-HIV-1 activities (hereafter termed *HIV-1 inhibitory antibodies*) can be elicited by current vaccine strategies, and numerous studies are exploring their roles in preventing HIV-1 acquisition. We review examples of strategies for eliciting potentially protective HIV-1 inhibitory antibodies.

Recent Findings—Heterologous prime-boost strategies can yield anti-HIV immune responses; although only one (canarypox prime, Env protein boost) has been tested and shown positive results in an efficacy trial (RV144). Although the immune correlates of protection are as yet undefined, the reduced rate of acquisition without a significant effect on initial viral loads or $CD4^+$ T cell counts, have raised the hypothesis of an RV144 vaccine-elicited transient protective B cell response.

Summary—In light of the RV144 trial, there is a critical need to define the entire functional spectrum of anti-HIV-1 antibodies, how easily each can be elicited, and how effective different types of antibody effector mechanisms can be in prevention of HIV-1 transmission.

Keywords

Vaccines; B-cells; Neutralizing Antibodies; Inhibitory Antibodies; Mucosal

Introduction

The central goal for induction of protective immune responses is to define strategies for routine elicitation of broadly neutralizing antibodies [1,2]. However, attempts to elicit HIV-1 broadly neutralizing antibodies by vaccination have been unsuccessful [3]. Conventional and successful non-HIV-1 vaccine approaches have relied on virus

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attenuation, although safety concerns halted these efforts for HIV-1. Thus, HIV-1 vaccine development is currently focused on safer strategies such as subunit immunogens and non-HIV-1 vectored inserts. Examples of HIV-1 immunogens under consideration for the elicitation of B cell responses are HIV-1 envelope protein and/or Env-expressing DNA and viral vectors. Attenuated virus vectors expressing HIV-1 genes (modified vaccinia Ankara (MVA), canarypox virus (ALVAC), adenovirus serotypes (Ad5, Ad26, Ad35), attenuated vaccinia strain (NYVAC), and Venezuelan equine encephalitis virus (VEE) have been utilized as single vector strategies or in prime-boost strategies. Recent HIV-1 envelope protein strategies have included diverse strains of gp120 or gp140 envelope proteins [4-6]. Heterologous prime boost strategies can be particularly effective at enhancing antibody responses over the prime alone [7] and when multi-valent envelope proteins are included as part of the immunogen, antibody responses against HIV envelopes from multiple clades can be elicited [5,7].

While the main goal of HIV-1 vaccine development is to define ways to induce broad neutralizing antibodies, other antibody HIV-1 inhibitory effects other than traditional neutralization are being considered as potentially protective. These inhibitory antibody activities include antibody ability to bind to virions and reduce movement of virions across mucus, the ability to inhibit movement of virions across epithelia [8], the ability to bind to Fc receptors and mediate a variety of Fc receptor mediated anti-HIV-1 activities including ADCC and ADCVI (reviewed in [9]), the ability to mediate β-chemokine release [10] that are important as soluble mediators in control of HIV infection [11]. Whether any of these HIV-1 inhibitory antibodies can prevent HIV-1 acquisition is unknown, but is a key question for the HIV-1 vaccine field. This review will focus on the types of B cell responses desired by HIV-1 vaccines and some of the current strategies employed for the induction and characterization of vaccine-induced B cell responses.

Next Steps after RV144 for Vaccine Elicited B Cell Responses

The Thai trial (RV144) consisted of a prime-boost vaccination of four doses of ALVAC HIV-1 (vCP1521) containing *env, gag, protease*) with an additional two doses of this vector combined with a gp120 protein boost (AIDSVAX B/E) that resulted in 31.2% efficacy in the absence of potent cross-clade neutralization breadth. [12]. The vaccine elicited binding antibody responses to the HIV-1 envelope in 99% of subjects as well as CD4 T cell responses in ~70% (Kim, J., Michael, N. *et al*, Keystone Symposium on HIV Vaccines, Banff, Canada 2010). The protection provided by RV144 was at the level of blocking acquisition of HIV-1 with no effect on viral load for those who became infected. Studies are ongoing to attempt to define the immune correlates of protection. In parallel with studies to define immune correlates, further plans for testing vaccines that can elicit, what are perceived to be better B cell responses, are underway. For example, the RV144 extended boost study, RV305, will examine the utility of additional vaccine boosts to enhance the durability of the vaccine elicited response. RV306, an intensive immunogenicity study, will examine the same vaccine regimen with a booster vaccination at 12 months to see if the durability of immune responses can be improved. Additionally, this protocol will be powered to understand host immune responses elicited through more extensive systemic and mucosal sampling. Importantly, since genetic factors, virus subtypes and route of transmission can impact elicited B cell responses, additional phase IIB studies are being considered to address these questions. In total, these additional studies with ALVAC and AIDSVAX will hopefully provide a better understanding of the immunogenicity of each of the vaccine components.

Heterologous Prime-Boost HIV-1 Vaccination

Unlike elicitation of antibodies to the hemagglutinin surface receptor by protective influenza vaccination [13], initial vaccine strategies based on HIV-1 monomers were unsuccessful. It remains to be determined why the B/E monomers failed to protect in the VAX003 and VAX004 studies [14,15], while the same B/E monomers used as a boost in the RV144 trial were associated with a measure of protection [12]. One potential contributor to the difference is the use of a heterologous prime-boost strategy. Thus, in other recent Phase I clinical studies [5-7,16] that have used heterologous prime-boost strategies or Env protein immunogens alone [4] and have elicited anti-Env binding antibodies, it will be important to determine whether inhibitory antibodies were elicited.

Vaccine Design for Better Exposure of HIV-1 Envelope Epitopes

The target of neutralizing antibodies is the HIV-1 trimer; however, use of the native trimer as an immunogen is difficult due to its instability. In addition to improving stability, effort is focused on greater exposure of Env epitopes known to be recognized by broadly neutralizing monoclonal antibodies.

Trimer Stabilization

Since recombinant Env trimers may be somewhat more immunogenic than Env monomers and the HIV-1 trimer can be structurally unstable, several approaches to stabilize the trimer have been attempted [17]. Dey *et al.* [18] demonstrated that structure-based stabilization of HIV-1 gp120 was able to enhance antibody responses against the coreceptor binding site. This study provided proof of concept that stabilization of conformational epitopes in Env can lead to enhanced immunogenicity.

CD4i Immunogens

Antibodies that bind to exposed and conserved epitopes arising from CD4 attachment are collectively called CD4-induced (CD4i) epitopes. Immunogens designed to elicit these types of antibodies could potentially elicit potent crossreactive antibodies [19,20]. A recent study using a SHIV challenge of macaques vaccinated with CD4i immunogens demonstrated that the induction of anti-CD4i antibodies corresponded with a lower viral load setpoint [21].

Virus-like Particles (VLPs) and Liposomes

Virus-like particles (VLPs) and liposomes are a safer alternative to live-attenuated viruses since they lack viral genomes but retain a virion-like membrane structure and can present surface HIV-1 trimers. VLPs have limitations similar to that of the native HIV-1 virion; paucity of trimers on the surface along with expression of non-functional forms of HIV-1 Env [22] that may limit their ultimate utility in HIV-1 vaccine design until these expression and structural hurdles can be overcome. However, recent success was achieved, in another infection model, with the elicitation of antibodies by chikungunya VLPs that provided protection from infection in macaques [23]. Liposomes are similar to VLPs in retaining a virion-like membrane structure, and can be engineered to express protein and or peptide immunogens along with adjuvants. Strategies involving Env subunits associated with lipids may be required for eliciting Env gp41 membrane proximal external region (MPER) antibodies. The broad neutralizing mAbs 2F5 and 4E10 require lipid binding in addition to gp41 MPER recognition for neutralization breadth [24,25]. Mutations in the MPER (such as L669S) [26] can enhance the exposure of the MPER and potentially may enhance the immunogenicity of such strategies.

Vaccine Designs to Overcome HIV-1 Diversity for Induction of Broad T Helper Responses

Computational methods have designed artificial viral proteins that provide optimal coverage of the diversity of circulating HIV-1 strains [27-29]. For example, two studies have demonstrated that the mosaic vaccine strategy elicited T cell helper responses and specific antibodies [30,31]. Strategies such as these that target optimal T cell responses, that include the elicitation of T helper cells, need to be examined as a component of vaccines that aim to elicit robust B cell responses. Further work in human clinical trials is needed to determine the breadth of the elicited immune responses and to understand how the conformation of these expressed proteins influence immunogenicity.

Engineering Immunity

Due to the difficulty in eliciting broadly neutralizing antibodies, approaches other than vaccination, are being explored to produce potent and broadly neutralizing anti-HIV antibodies using 'engineering immunity' to directly provide the antibody genes *in vivo*. One strategy to program human B cells used autologous human hematopoietic stem/progenitor cells (HSPCs) transduced with the b12-IgG1 gene for differentiation into antibody secreting cells [32]. Another recent study using adeno-associated virus gene transfer of SIV specific antibodies into macaques demonstrated protection and maintenance of neutralizing antibody responses [33]. Although not tested in human clinical trials, these studies do represent alternative strategies for the delivery of preexisting neutralizing antibodies for protection from HIV-1 transmission. Passive infusion of neutralizing antibodies have shown protection in nonhuman primate models [34,35] and suggest that approaches that provide preexisting neutralizing antibodies could potentially be protective.

Assessing B Cell Responses to HIV-1 Vaccination

Vaccine-elicited immune responses constituting protective immunity against HIV-1 infection are not yet delineated. However, the goals of preventive vaccine studies are to identify immunogens and vaccine strategies capable of eliciting the highest levels and broadest specificities of cellular and humoral responses. An assay currently standardized and utilized around the world for assessing vaccine elicited neutralizing antibodies is the TZM-bl assay, wherein diverse viruses of multiple genetic subtypes are used for the assessment of neutralization breadth [36]. Additional types of neutralization assays [37] are also being utilized. And further studies are aimed at understanding how to inhibit various stages of the mucosal transmission event (inhibition of virion migration through mucus [38], virus aggregation [39], complement mediated virolysis [40,41], virus capture[42,43], IgA-mediated neutralization [44,45], traditional virus neutralization [36,37,46,47], and/or inhibition of virus transcytosis [8,48,49], intraepithelial virus neutralization[50], Fcγ-receptor mediated anti-HIV-1 activity [51]including, antibody dependent cellular cytotoxicity (ADCC) [4,52] and antibody dependent cellular viral inhibition (ADCVI) [53], inhibition of macrophage infection [37,54] and induction of anti-HIV-1 innate immune responses [10,11] (Table 1). Thus, a broad range of anti-HIV-1 antibody assays will be needed for evaluation of antibodies induced by current and future vaccine candidates, in order to determine which type of induced antibodies can prevent HIV-1 transmission.

Antigen Specific B Cell Isolation, Single Memory B Cell Cultures and MAb Generation

Broadly reactive neutralizing antibodies in complex with the envelope regions involved in recognition of the viral envelope have been examined by X-ray crystallography. Analyses of

these structures can provide detailed information of the envelope neutralization epitopes, such as recently shown for CD4bs antibodies that had divergent neutralization profiles due to the slight differences in the bound conformation of the mAbs [55]. New work, by several laboratories, has rapidly expanded the number of broadly neutralizing mAbs that hopefully will indicate new targets for a vaccine.

Recent advances have been made in isolating single Env-specific B cells by either single cell sorting by flow cytometry or from memory B cell cultures coupled with high-throughput neutralization screening assays of B cell supernatants [56]. Among the new antibodies generated with these technologies, mAbs PG9 and PG16 recognizing conserved regions of the variable loops in gp120 are potent and broadly-reactive against approximately 73-79% of HIV-1 strains [57]. Furthermore, two newly identified CD4bs bNAb VRC01 [58] and HJ16 [59] are more potent than previously described CD4bs antibodies. These newly identified mAbs from antibodies naturally elicited in humans will provide insights into how the immune response can recognize the viral envelope to generate a potentially protective response.

Functional Roles of HIV-1 Inhibitory Antibodies

HIV-1 transmission occurs predominately through mucosal surfaces; thus vaccine strategies that can elicit mucosal antibodies with antiviral properties may prove to be critical. In addition to traditional HIV-1 neutralization neutralizing, antibodies may also aggregate virions, inhibit movement through cervical mucus, inhibit transcytosis, and/or mediate Fc receptor mediated antibody inhibition. In the initial stages of transmission in the female genital tract, HIV-1 must traverse the mucus layer and then cross the epithelial cell barrier. Vaccine elicited antibodies that can block these events near the time of transmission might be among the most efficacious types of antibodies [60]. In the female genital tract, inhibition of movement through the cervical mucus layer may provide a layer of protection against HIV-1 [38], particularly if anti-HIV antibodies are present. Aggregation of virus [39], inhibition of virion transcytosis, and intraepithelial neutralization of virions may also be components of an effective antibody response.

In addition to direct binding to antigen to neutralize virus entry, effector functions of antibodies can depend on the interaction of antibodies with Fc receptors on B cells, NK cells, dendritic cells, neutrophils and monocyte-macrophages (reviewed by [9]). Antibody-dependent cell-mediated virus inhibition was shown to be associated with protection in a neutralizing antibody passive protection study in non-human primates [61]. Moreover, inhibitory antibodies that depend on Fc receptor mediated functions, such as antibody dependent cellular cytotoxicity (ADCC), may be found in cervicovaginal fluids in natural infection [62] and in plasma in HIV-1 elite controllers. Whether current vaccine strategies can elicit these types of inhibitory antibodies and whether they may be the surrogate or correlate of protection in the RV144 trial will be important to determine.

Surrogates of HIV-1 Inhibitory Antibody Function: IgG Subclasses, IgA Specificities and Antibody Avidity

The measurement of HIV-1 specific binding antibodies in vaccine trials can be pivotal for the initial assessment of vaccine immunogenicity and for understanding which vaccines will elicit potentially protective antibodies. Antibody IgG subclasses (Ig1, IgG2, IgG3, IgG4) [63,64] and isotypes (IgA) [65] measured in response to vaccination can provide information on the quality of the B cell response to vaccination. IgG1 and IgG3 are the most functional of the subclasses in that they have been associated with HIV-1 neutralization, complement fixation, FcR binding and ADCC/ADCVI (reviewed in [66]). IgA antibodies at mucosal

sites have been correlated with protection in exposed uninfected subjects [67,68]. Measurement of mucosal antibody responses and antigen specific plasma antibody subclasses in vaccine trials may be help define immune correlates of protection.

Maturation of HIV-1 specific antibodies includes an increase in avidity to the epitope of recognition. Thus, avidity measurement of antibody-antigen interactions may be useful to predict functional antibody responses such as ADCC/ADCVI and neutralizing antibodies and could be a surrogate for a B cell response that impacts viral load or prevent acquisition. Correlations between anti-Env antibody avidity and peak postchallenge viremia was found in two recent non-human primate immunization studies [69,70] (both which have immunogen components similar to that being used in ongoing human clinical trials). Moreover, in another NHP study, ADCC and ADCVI were directly correlated with avidity [71]. There is precedence for predicting antibody function with avidity measurement, since antibody avidity in pneumococcal [72] and hepatitis B vaccination [73] was linked to protective immunity. Thus, further studies are needed to determine if antibody avidity for Env or virion binding will associate with protection from HIV-1 transmission in human clinical trials.

Memory B cells and Durability of Response

Long-lived antibody responses are a hallmark of successful vaccines (i.e., tetanus, diphtheria, yellowfever) and are maintained by bone marrow plasma cells. In contrast, in HIV-1 infection anti-envelope antibody level half-lives were 33–81 wk in plasma from antiretroviral drug-treated HIV-1^+ subjects [74]. However, antibody titers against influenza did not decay in-between yearly or biennial influenza vaccine boosts in the same patients and antibody responses to HIV-1 Gag were more durable. This work demonstrated that HIV-1 envelope induces predominantly short-lived memory B cell-dependent plasma Abs in HIV-1 infection and also by HIV-1 envelope vaccination. Consistent with a rapid half-life of the anti-HIV-1 Env antibody response, the immunity provided by RV144 appeared to also decline over time. Thus, a significant barrier to the development of a successful vaccine is the ability to generate sufficient titers of long-lived anti-Env antibody responses. However, the definition of sufficient titer will be key and provides some hope that if a high enough titer is elicited initially, despite the half life, an adequate amount of specific antibody will be present for a considerable duration (although boosting may always be required to raise the levels after a period of time). For example, in several recent human vaccine protocols [4,75,76], antibody responses many weeks post the last vaccination have been detected suggesting that memory B cell responses developed. Analyses of the kinetics and strength of this durable antibody response in addition to the use of antigen-specific memory B cell assays [77] and an analysis of the discordance between memory B cell and circulating anti-Env antibody responses [78] could enable more informed vaccine design for the elicitation of stronger memory responses to the HIV-1 envelope. Probing the potential correlation between a strong B cell responses and CD4 help will garner more attention as more vaccines elicit both potent antibody and CD4 responses.

Conclusions

For prevention of HIV-1 transmission by vaccination, humoral immunity to HIV-1 via pre-existing or rapidly elicited antibodies with specificity to the transmitted/founder HIV-1 is likely central to a protective response. Multiple antibody-mediated effector functions (e.g. virus aggregation, FcR anti-HIV-1 functions) other than traditional neutralizing antibodies can potentially be generated by different vaccine strategies. Further analyses on potential correlations between antibodies with diverse antiviral functions and protection from HIV-1 acquisition in humans are warranted. Deciphering which HIV-1 inhibitory antibodies are

most easily elicited by current vaccines and which are most efficacious is a critical goal of current HIV-1 vaccine research.

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Table 1

Inhibitory Functions of anti-HIV Antibodies

Target	Site of Antibody Block*	Recent References (Human Studies)
Virions (Cell Free)	Mucus Inhibition	[38]
	Virion Aggregation	[39]
	Complement mediated Virolysis	[40,41]
	Virus Capture	[42,43]
	IgA Mediated Neutralization	[44,45]
	Traditional Virus Neutralization	[36,37,46,47]
Infected Cells (Cell Associated Virus)	Inhibition of Transcytosis	[8,48,49]
	Intraepithelial Neutralization	[50]
	Fcγ-receptor (FcγR)- mediated:	[51]
	ADCC	[4,52]
	ADCVI	[53]
	Macrophage Neutralization	[37,54]
Trigger of Innate Immunity	Release of β-chemokines	[10,11]

*Note: Some of the assays may inherently measure multiple steps of antibody blocking.